Molecular Ecology Notes (2006) **6**, 1147–1149 doi: 10.1111/j.1471-8286.2006.01466.x

PRIMER NOTE

Isolation and characterization of 10 polymorphic microsatellites in saltcedars (*Tamarix chinensis* and *Tamarix ramosissima*)

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Abstract

***Tamarix ramosissima* and *Tamarix chinensis* are invasive weed species in western North America. Previous studies based on single locus DNA sequence data revealed some information about the invasion process, but multilocus markers can provide additional information about levels of introgression and genotype origins. We have developed primers that amplify 10 polymorphic microsatellite loci from *T. ramosissima*; these primer pairs also successfully amplify polymorphic microsatellites from the closely related *T. chinensis*, a species that forms hybrids with *T. ramosissima* in the western USA.**

Keywords: invasive, saltcedar, Tamaricaceae, *Tamarix*, weed

Received 29 January 2006; revision received 19 February 2006; accepted 24 May 2006

The Old World plant genus *Tamarix* L. (family Tamaricaceae) contains about 54 species of shrubs and trees (Baum 1978). Multiple *Tamarix* species were brought to the USA from southern Europe and Asia during the 1800s to be used for shade and erosion control (Baum 1967). Western USA floodplains, riverways, wetlands, and lake margins now contain large-scale invasions totalling 470 000–650 000 ha (Zavaleta 2000) of *Tamarix ramosissima* Ledeb. and *Tamarix chinensis* Lour. (both commonly named 'saltcedar' or 'tamarisk'). Both saltcedar species are diploid with $2n = 24$ (Zhai & Li 1986) and form hybrids which are common in the invasion (Gaskin & Schaal 2002).

Leaf tissue obtained from *T. ramosissima* (*Gaskin 4173.43*, Howery Island, MT) was silica-dried and genomic DNA was extracted using the DNeasy kit (QIAGEN). DNA fragments containing microsatellites were isolated by the biotinylated-oligonucleotide capture method of Reddy *et al.* (2001), with minor modifications. Briefly, a 2-μg sample of genomic DNA was digested for 3 h in a single reaction mixture containing restriction endonucleases *Hae*III, *Rsa*I and *Dra*I (20 U each), resulting in a diverse population of blunt-ended restriction fragments with an average size of ± 750 bp. Fragments were ligated to the double-stranded adaptor molecule AP11/12, which consists of AP11 (5′-CTCTTGCTTAGATCTGGACTA-3′) and AP12 (5′-pTAGTCCAGATCTAAGCAAGAGCACA-3′, where p = 5′ phosphate), using 30 Weiss U of T4 DNA Ligase for 16 h at 14 °C. Ligation products were pre-amplified in 10 cycles of polymerase chain reaction (PCR) using the single primer AP11. Approximately 100 ng of the pre-amplified product was subjected to capture using the biotinylated oligos $b(GA)_{18}$, $b(CA)_{18}$, $b(ACA)_{14}$, $b(CAT)_{14}$ and $b(AGA)_{14}$ (b = 5′ biotinylation). Final stringency washes were performed in 3× SSC, 0.1% SDS at 60 °C. Captured fragments were subjected to 30 additional cycles of PCR using primer AP11, then cloned into the plasmid vector pCR-Blunt II-TOPO (Invitrogen). Ninety-six clones were sequenced in both directions using the primers T7 (5′-TAATACGACTCACTATAGGG-3′) and VectorR2 (5′-GAGCTCGGATCCACTAGTAACG-3′). Sequencing reactions were purified using Centrisep columns (Princeton Separations) and run on a CEQ 2000XL (Beckman) sequence analyser using the manufacturer's protocols. Of the 96 clones, 52 contained repetitive fragments. We designed primer sets for 30 clones using the PRIMER 3 program (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi).

To test for polymorphism, genomic DNAs from eight geographically diverse individuals were extracted using a cetyltrimethyl ammonium bromide method. PCR amplifications

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Table 1 Characterization of 10 microsatellites isolated from *Tamarix ramosissima*. The observed (H_O) and expected (H_E) heterozygosities and *P*-value of an exact HW test (HWE) are listed for: native *T. ramosissima* ($n = 6$); native *T. chinensis* ($n = 6$); and USA invasion specimens ($n = 40$). Also listed are the number of alleles (N_a) the annealing temperature (T_a) used in the polymerase chain reaction

Locus	Samples included	Core repeat (cloned allele)	Size range (bp)*	N_a	H_O	H_E	HWE	T_a (°C)	Primer sequence (5′–3′)	GenBank Accession no.
T1B8	*T. ramosissima*	$(AC)_6(GC)_2(AC)_{12}$	353–401	9	1.00	0.94	1.000	60	F: CGTTAGCAGGTTGGACATGA†	DQ233496
	T. chinensis		333–347	3	1.00	0.68	0.090		R: TTTGAGTGTCAGTCGATGGTG	
	USA invasion		327–381	11	0.83	0.81	0.412			
T1B9	*T. ramosissima*	$(CTT)_7$	273–285	4	0.17	0.68	0.004	60	F: TCCGCCTCTCTTTCTGTCAT†	DQ233494
	T. chinensis		279	1	0.00	0.00	–		R: CGCCAAGAACCACAATTTTT	
	USA invasion		279–291	4	0.45	0.62	0.028			
T1E1	*T. ramosissima*	$(GT)_{17}$	217–241	8	1.00	0.92	1.000	60	F: ATTACGACCTGCAAGCATCC†	DQ233495
	T. chinensis		229–233	2	0.17	0.17	1.000		R: AATCGAATGCCTCGTGACTT	
	USA invasion		217–245	9	0.68	0.81	0.320			
T1G6	*T. ramosissima*	$(GT)_3T(GT)_8$	312–326	7	0.83	0.87	0.860	56	F: CAACCCAACGTGTCACAGTC†	DQ233493
	T. chinensis		320–322	2	0.00	0.30	0.090		R: TCCAAACATAAATCGGGTCAA	
	USA invasion		314–360	5	0.55	0.77	0.004			
T1C7	*T. ramosissima*	$(CAA)_3(CAT)_2(CAA)_5$	322–325	2	0.00	0.48	0.030	58	F: TAAGGGTGGGAATGTCTTGG†	DQ233492
	T. chinensis		322–325	2	0.33	0.30	1.000		R: TTGTTTGGGATAATTTTTGGA	
	USA invasion		304–322	3	0.08	0.17	0.005			
T1G9	*T. ramosissima*	$(ATC)_9$	387–414	6	0.83	0.76	1.000	58	F: CCATAAGTGCCCCATCAAAG†	DQ233491
	T. chinensis		393–396	2	0.83	0.53	0.394		R: AAAAGCTTTCCCCAAATACCA	
	USA invasion		369–405	7	0.26	0.53	0.000			
T1C10	*T. ramosissima*	$(AC)_{10}$	321–331	6	0.33	0.87	0.003	63	F: AACGAGGATCATGAAAAGGA†	DQ233497
	T. chinensis		323–331	3	0.33	0.54	0.032		R: GACACATGTCCCTACCATTGAA	
	USA invasion		317–343	6	0.38	0.57	0.000			
T1G11	*T. ramosissima*	$(AG)_{10}AA(AG)_4AT(AG)_9$	149–183	5	0.67	0.82	0.202	58	F: AAGCTCCATGCTTGCTTCAT†	DQ233499
	T. chinensis		177–181	3	0.17	0.59	0.031		R: GACCATTGATATGCCCCAAT	
	USA invasion		171–183	4	0.38	0.59	0.001			
T1C1	*T. ramosissima*	$(AC)_{11}$	239–247	2	0.40	0.36	1.000	54	F: GAGGCAAGCCTCTTGAAATG	DQ233498
	T. chinensis		249–251	2	0.40	0.45	1.000		R: TGTGCTGCCGTCTATTTCTC	
	USA invasion		239–251	4	0.55	0.59	0.383			
T1D12	*T. ramosissima*	$(AC)_8$	562–580	3	0.33	0.73	0.200	52	F: GGATTTCCCTCAAAGCACAA†	DQ233500
	T. chinensis		574–596	4	1.00	0.76	0.325		R: TTCCCCCACTTGTAATGAGC	
	USA invasion		558–604	7	0.76	0.79	0.183			

*Length includes 19 bp when M13-tailed primer used.
†M13-tail added to 5′ end of forward primer.

were performed using 10 μL reaction volumes with 1× Biolase PCR buffer (Bioline), 0.2 mM of each dNTP, 2.5 mM $MgCl_2$, 0.2 μM of each primer, and 0.5 U Biolase *Taq* polymerase (Bioline). Samples were amplified with the following PCR protocol: denaturation at 94 °C for 2 min; 30 cycles at 94 °C for 30 s, annealing temperature (Table 1) for 1 min, 72 °C for 2 min; one step of 72 °C for 5 min. PCR products were initially visualized on a 3% agarose gel stained with ethidium bromide. Of the 30 primer pairs developed, 11 did not produce an amplification product and seven amplified multiple products (suggesting possible nonspecific binding), and were dropped. The remaining 12 primer pairs gave robust and readily interpretable amplification products. These 12 primer sets were then screened against 52 *T. ramosissima* and *T. chinensis* specimens, including six native *T. ramosissima* from Asia, six native *T. chinensis* from eastern China, and 40 saltcedars (either *T. ramosissima*, *T. chinensis*, or hybrids; determination to species is difficult because of morphological similarity of the two species and past introgression). In these analyses, one forward primer was dye-labelled on the 5′ end, and for the rest of the forward primers we added a 19-bp M13 tail (5′-CAC-GACGTTGTAAAACGAC-3′) to the 5′ end, as in Schuelke (2000). Only a single dye was used: WellRED D4-PA (Proligo). Dye-labelled PCR amplifications were performed as above, but with 0.1 μM of the forward M13-tailed primer and 0.2 μM each of the reverse and dye-labelled M13 primers (for the T1C1 locus 0.2 μM each of a dye-labelled forward primer and a reverse primer were used). Samples were amplified with the following PCR protocol: denaturing at 94 °C for 3 min; 30 cycles at 94 °C for 30 s, annealing temperature (Table 1) for 30 s, 72 °C for 30 s; eight cycles at 94 °C for 30 s, 51 °C for 30 s, 72 °C for 30 s; and one step at 72 °C for 10 min: A total of 0.2 μL of each PCR product was

combined with 0.3 μL of 600 bp size standard and 29.5 μL of de-ionized formamide and loaded into a CEQ 2000XL fragment analyser using the manufacturer's protocols. Two primer pairs produced monomorphic fragments, and the remaining 10 were polymorphic (Table 1). Observed and expected heterozygosity, deviation from Hardy–Weinberg equilibrium (HWE), and linkage disequilibrium were all calculated using GENEPOP version 3.4 (Raymond & Rousset 1995). GenBank accession numbers are included in Table 1.

Six of the 10 loci exhibited significant deviations from HWE ($P < 0.05$) in the USA invasion, specifically a deficiency of heterozygotes, which is consistent with the presence of null alleles, inbreeding, or population subdivision. Significant linkage disequilibrium was found among 21 of the 45 (47%) locus pairs ($P < 0.05$ using Fisher's method). Thirty-three of the 52 plant samples were from one region along the Green River in Utah (spanning approximately 60 km), and their close geographical relationship may have influenced linkage disequilibrium for these loci. We then tested a subset of the above plants from both species across their native and invasive ranges ($n = 15$) and found only three pairs of loci (7%) that exhibited significant linkage disequilibrium. Collectively, these 10 microsatellite loci exhibit much more variation than was available in the single DNA sequence locus used in previous studies, and they will be useful for analysing levels of introgression, geographical origins, and population structure of the saltcedar invasion.

Acknowledgements

We thank D. Cooper, A. Birken, and G. Yatskievych for collection of samples. This research was made possible in part by financial support from the USA Department of the Interior, Bureau of Land Management and Bureau of Indian Affairs.

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